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Normohomocysteinaemia and vitamin-treated hyperhomocysteinaemia are associated with similar risks of cardiovascular events in patients with premature peripheral arterial occlusive disease. A prospective cohort study

S. C. DE JONG[1,2], C. D. A. STEHOUWER[1,3], M. VAN DEN BERG[1,2], T. W. GEURTS[2], L. M. BOUTER[4] & J. A. RAUWERDA[1,2]

From the [1]Institute for Cardiovascular Research, Vrije Universiteit; [2]Department of Surgery, Division of Vascular Surgery, Academisch Ziekenhuis Vrije Universiteit; [3]Department of Internal Medicine, Academisch Ziekenhuis Vrije Universiteit; and [4]Department of Epidemiology and Biostatistics, Vrije Universiteit, Amsterdam, The Netherlands

Abstract. de Jong SC, Stehouwer CDA, van den Berg M, Geurts TW, Bouter LM, Rauwerda JA (Institute for Cardiovascular Research; Division of Vascular Surgery, Academisch Ziekenhuis; Department of Internal Medicine, Academisch Ziekenhuis; and Department of Epidemiology and Biostatistics, Vrije Universiteit, Amsterdam, The Netherlands) Normohomocysteinaemia and vitamin-treated hyperhomocysteinaemia are associated with similar risks of cardiovascular events in patients with premature peripheral arterial occlusive disease. A prospective cohort study. *J Intern Med* 1999 **246**: 87–96.

Objectives. Mild hyperhomocysteinaemia (HHC), fasting or after methionine loading, is associated with an increased risk and severity of atherosclerotic vascular disease. Post-methionine and fasting HHC are responsive to treatment with vitamin B_6 and folic acid. We performed a prospective cohort study amongst normohomocysteinaemic and vitamin-treated (vitamin B_6, 250 mg plus folic acid, 5 mg daily) hyperhomocysteinaemic patients with premature peripheral arterial occlusive disease and assessed the incidence of cardiovascular events.

Design. We studied 273 consecutive patients with clinically manifest peripheral arterial occlusive disease with onset before the age of 56, 79 (28.9%) of whom had postmethionine HHC. Follow-up was obtained in 232 (85%) patients. At baseline, 70 (30%) were hyperhomocysteinaemic after methionine loading and started treatment with vitamin B_6 and folic acid; 162 (70%) were normohomocysteinaemic (reference group).

Results. During the follow-up period (median 20, range 1–63 months), 48 (29.6%) and 23 (32.9%) of the normo- and the hyperhomocysteinaemic patients, respectively, had a new cardiovascular event. Most (75%) involved the peripheral arterial system. The crude incidence rate for any cardiovascular event was 0.16 (95% CI, 0.12–0.21) per person per year in the normohomocysteinaemic and 0.16 (95% CI, 0.09–0.22) per person per year in the hyperhomocysteinaemic group.

Multivariate Cox regression analyses showed that higher plasma homocysteine levels were associated with an increased risk of new cardiovascular events in the normohomocysteinaemic patients (relative risk [RR] per 1 μmol L^{-1}, 1.17 [CI, 1.05–1.30] for fasting and 1.06 [CI, 1.01–1.12] for postmethionine levels), but not in the hyperhomocysteinaemic (vitamin-treated) patients. The adjusted RR for new cardiovascular events in the hyper- as compared to the normohomocysteinaemic patients was 0.76 (CI, 0.33–1.74).

Conclusions. These data are consistent with a protective effect of treatment with vitamin B_6 and folic acid in patients with premature peripheral arterial occlusive disease and postmethionine HHC. Double-blind randomized trials are necessary to confirm this.

Keywords: cardiovascular events, mild hyperhomocysteinaemia, peripheral arterial occlusive disease, prospective study, vitamin treatment.

Introduction

Mild hyperhomocysteinaemia (HHC), fasting or after methionine loading, is associated with an increased risk of premature vascular disease [1–5]. Mild HHC after methionine loading is found in a substantial percentage of these patients, varying from 33% in patients with peripheral to 15% in patients with coronary arterial occlusive disease [6–9]. In addition, several studies have found relations between the severity of atherosclerotic vascular disease and homocysteine concentrations [10–12], including postmethionine levels [10].

As in severe homocystinuria, treatment of HHC with cofactors or cosubstrates of homocysteine metabolism can lower homocysteine levels [7, 8, 13]. For example, postmethionine and fasting total homocysteine (tHcy) levels were normalized after treatment with 250 mg vitamin B_6 plus 5 mg folic acid in, respectively, 92 and 91% of vascular patients with HHC [7]. Such treatment was associated with a decrease in elevated plasma levels of endothelium-derived proteins, which are thought to reflect endothelial dysfunction [14]. These results suggest that a reduction in tHcy levels is associated with amelioration of endothelial dysfunction and by this means may influence the course of atherothrombotic disease.

Patients with peripheral arterial occlusive disease have an increased morbidity and mortality, which is generally due to accompanying cardiovascular disease [15, 16]. Furthermore, patients with even slightly elevated tHcy levels are more likely to demonstrate clinical progression of peripheral and coronary artery disease than patients with normal tHcy levels [17, 18]. It is not known whether homocysteine-lowering treatment will influence the clinical outcome of patients with peripheral arterial occlusive disease who also have HHC. To investigate this, we initially wished to perform a placebo-controlled, randomized clinical trial of the effects of vitamin B_6 and folic acid on cardiovascular outcomes, but this was not acceptable to the hyperhomocysteinaemic patients, who indicated that they strongly preferred treatment with B-vitamins. We therefore chose to perform a prospective cohort study amongst normohomocysteinaemic and vitamin-treated hyperhomocysteinaemic patients with premature peripheral arterial occlusive disease.

Methods

Patients

In January 1991, we started a HHC screening programme amongst consecutive patients who had been directly referred by their general practioner to the Department of Surgery, Division of Vascular Surgery of the Academisch Ziekenhuis, Vrije Universiteit in Amsterdam, the Netherlands. All patients with a history of clinically manifest peripheral arterial occlusive disease with onset before the age of 56 years underwent a methionine loading test (see below). Peripheral arterial occlusive disease was defined by the presence of intermittent claudication confirmed by an ankle/brachial index <0.9 at rest and/or a decrease of >0.15 of the index after treadmill exercise testing, or ischaemic rest pain confirmed by a resting ankle pressure below 40 mmHg, gangrenous ulcers diagnosed by the presence of a nonhealing ulcer confirmed by a resting ankle pressure below 60 mmHg, or amputation for ischaemia.

From January, 1991, to June, 1995, we thus screened 273 patients, of whom 35 (12.8%) had fasting and 79 (28.9%) had postmethionine HHC. Twenty-four patients had both fasting and postmethionine HHC. All 79 patients with postmethionine HHC were treated with vitamin B_6 (250 mg) plus folic acid (5 mg). All patients were then followed routinely on a yearly basis or earlier when clinically indicated. At the yearly visit, we specifically reviewed the occurrence of new cardiovascular events and the progression of peripheral arterial occlusive disease. In case of new symptoms or progression of peripheral arterial occlusive disease, noninvasive vascular tests were performed by technicians not aware of other clinical and laboratory data. Seventeen patients were lost to follow-up because they had moved to another area and could not be contacted; four patients refused permission for the prospective follow-up. These 21 patients were excluded. Thus, 252 patients who had given written informed consent were included. The study was approved by the local ethics committee.

Methionine loading test and laboratory analyses at baseline

After an overnight fast, venous blood samples were taken between 09.00 and 10.00 hours. Patients were asked to refrain from smoking and from using alcohol from 22.00 hours on the evening prior to blood sampling. A second blood sample was obtained 6 hours after an oral methionine load (0.1 g kg^{-1} body weight). Plasma samples were stored at − 30 °C until use. Plasma levels of tHcy were determined within 1 week of blood sampling. tHcy (free plus protein bound) concentrations were measured by using tri-*n*-butylphosphine as the reducing agent and ammonium 7-fluorobenzo-2-oxa-1,3-diazole-4-sulphonate as the fluorochromophore, followed by high-pressure liquid chromatography (HPLC) with fluorescence detection [19]. Reference values for fasting and postmethionine tHcy levels in our laboratory are <18 and <54 μmol L^{-1} in men, <15 and <51 μmol L^{-1} in premenopausal women, and <19 and <69 μmol L^{-1} in postmenopausal women [10]. These reference values were obtained from a group of vitamin B_6, B_{12}, and folic acid-replete healthy volunteers: mean age (SD) for men (n = 23) and women (n = 41 pre- and n = 27 postmenopausal), 36.9 (5.7) and 42.3 (9.5), respectively. Patients who, on the basis of these reference values, were hyperhomocysteinaemic after methionine loading (n = 79) started treatment with vitamin B_6 (250 mg) plus folic acid (5 mg). Eleven patients had fasting HHC (18.2 ± 2.2 μmol L^{-1}) without postmethionine HHC. They did not receive vitamin treatment. After 12 weeks of treatment, the postload tHcy concentration was within the reference range in all patients.

At the time of the methionine loading test, we recorded baseline data, i.e. age, body weight and height, body mass index (the weight in kilograms divided by the square of the height in metres), menopausal status (postmenopause was defined as absence of menstrual bleeds >1 years), current and past smoking habits, use of medication (including aspirin and oral anticoagulants), history of physician-diagnosed diabetes, hypertension and/or hypercholesterolaemia, and blood pressure (measured after 15 min of supine rest). Diabetes and hypertension were defined according to WHO criteria; hypercholesterolaemia as serum total cholesterol >6.5 mmol L^{-1} and/or the use of cholesterol-lowering medication. A detailed history was taken with regard to angina pectoris, myocardial infarction, transient ischaemic attack and stroke [10].

Just prior to the methionine loading test, venous blood samples were taken for measurement of serum lipids (total and high density lipoprotein [HDL] cholesterol and triglycerides [enzymatically]). Low density lipoprotein [LDL] cholesterol was calculated by Friedewald's formula [20]. We also measured serum levels of creatinine (modified Jaffé reaction), vitamin B_6 (measured as pyridoxal-5-phospate by HPLC with fluorescence detection after precolumn derivatization with semicarbazide; reference, >17 nmol L^{-1}), vitamin B_{12} (radioassay, Becton Dickinson, France; reference, >80 pmol L^{-1}), and folate (radioassay, Becton Dickinson; reference, >3.4 nmol L^{-1}).

Follow-up and identification of new cardiovascular events

Each patient's duration of follow-up, in months, was defined as the time between the performance of the initial methionine loading test (baseline) and the occurrence of the first new cardiovascular (coronary, peripheral or cerebral arterial) event, or April 1st, 1996. If data from the hospital files were not clear, the patient's general practitioner was contacted for clarification. New coronary arterial disease was defined as myocardial infarction and newly diagnosed (WHO clinical definitions) or progressive angina pectoris (reduction of exercise tolerance estimated by a change in stage as defined by the New York Heart Association, and/or unstable angina, in all cases confirmed by exercise electrocardiographic stress testing and/or angiography). New peripheral arterial disease was defined as new intermittent claudication (defined as intermittent claudication in the other limb from that in which peripheral arterial occlusive disease had initially been present, confirmed by an ankle/brachial index <0.9 at rest and/or a decrease of >0.15 of the index after treadmill exercise testing), or as progression of peripheral arterial occlusive disease, defined as progression of clinical symptoms: (i) new symptoms of intermittent claudication in the same limb after initially successful treatment by reconstructive peripheral surgery or transluminal angioplasty (PTA); (ii) a (subjective) decrease in the walking

distance; (iii) occurrence of critical ischaemia, defined by the occurrence of ischaemic rest pain or gangrenous ulcers and confirmed by a resting ankle pressure below 60 mmHg; (iv) acute arterial thrombosis. A diagnosis of progression of clinical symptoms was accepted *only* when confirmed by a decrease in ankle/brachial index at rest of at least 0.15, and/or angiographically. New cerebral artery disease was defined as transient ischaemic attack or stroke (WHO clinical definitions). All diagnoses were accepted only when corroborated by a written report by a physician.

Of the 252 patients initially included, a further 20 were excluded from all analyses because data from the hospital files were unclear or missing and no additional information could be obtained from the general practitioner. A total of 232 (85%) patients were thus included; at baseline, 70 (30%) had hyperhomocysteinaemia and 162 (70%) normohomocysteinaemia after methionine loading (Fig. 1). Compared to those included, the excluded group (n = 41) did not differ significantly with regard to the prevalence of the major cardiovascular risk factors, i.e. male sex, cardiovascular history, smoking, hypertension, hypercholesterolaemia, diabetes mellitus, and hyperhomocysteinaemia (data not shown).

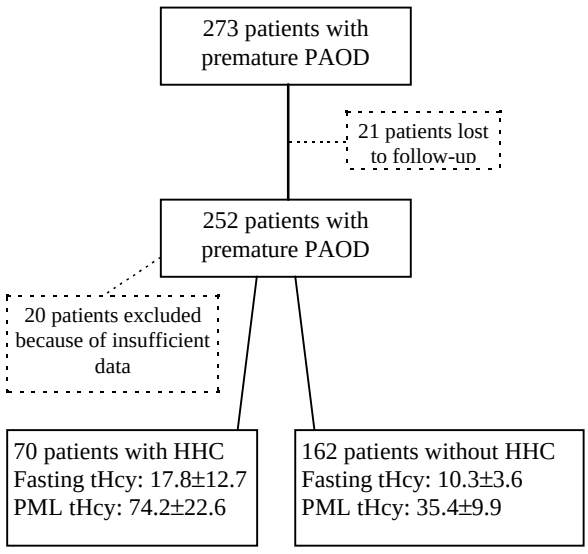

Fig. 1 Flow diagram of patient population.(PAOD, peripheral arterial occlusive disease; PML, postmethionine loading; HHC, hyperhomocysteinaemia).

Data analyses

Data were analysed using the statistical package SPSS for Windows 6.1 (Chicago, Il, USA). Descriptive data are given as mean (SD). Skewed data were logarithmically transformed. Continuous variables were compared by Student's t-test (for means) and percentages by chi-square tests.

We assessed the incidence of cardiovascular events in the follow-up period. We then calculated crude incidence rates of cardiovascular events in the entire group and separately for the patients with and without postmethionine HHC. Our primary interest was to analyse whether the presence of vitamin-treated postmethionine HHC was related to the incidence rate of cardiovascular events. Cox′ regression models were used for testing the association between the incidence of cardiovascular events on the one hand and possible prognostic baseline covariates on the other, i.e. age, sex, menopausal status, previous coronary and/or cerebral artery disease, presence of hypercholesterolaemia, hypertension and diabetes, current or past smoking and number of pack years (pack years were calculated by multiplying the number of cigarette packages smoked per day by the number of years the patient smoked), body mass index, use of aspirin or oral anticoagulants, systolic and diastolic blood pressure, serum lipids (total, HDL and LDL cholesterol, and triglyceride), serum vitamin B_6, B_{12} and folate, serum creatinine, presence of fasting or postmethionine HHC, and plasma tHcy concentrations (fasting, postmethionine and delta [defined as the difference between postmethionine and fasting tHcy level]). For the patients with postmethionine HHC, we considered both their initial tHcy levels and those after treatment. Covariates that had a P-value of <0.2 when considered as a prognostic risk factor in isolation were selected for multivariate Cox-regression analyses. Presence of HHC (yes/no) and plasma tHcy level were never included together in the same multivariate analysis.

All relative risks (RRs) are given with their 95% confidence interval (CI) in parentheses and contrast the risk in the presence versus the absence of the relevant risk factor (e.g. presence versus absence of hypertension or HHC) or are expressed per unit of

that risk factor (e.g. 1 mmHg for diastolic blood pressure and 1 μmol L^{-1} for plasma tHcy level). All testing was two-tailed with 0.05 as the level of significance.

Results

Table 1 shows clinical characteristics of the 232 patients at baseline. Of the patients with postmethionine HHC, 31.4% (22 out of 70) had a history of coronary and/or cerebral artery disease, compared to 25.5% (33 out of 162) of the normohomocysteinaemic patients ($P = 0.08$). After treatment of the patients with postmethionine HHC with vitamin B_6 plus folic acid, their fasting tHcy levels were lower than the fasting levels in the patient group without HHC ($P = 0.01$). However, the increase in tHcy levels (delta) after treatment, although within the reference range, remained higher in the patients with postmethionine HHC ($P = 0.01$).

During the follow-up period (median 20; range 1–63 months), 48 (29.6%) and 23 (32.9%) of the patients with postmethionine normo- and hyperhomocysteinaemia, respectively, had a new cardiovas-

Table 1 Baseline clinical and laboratory characteristics of 232 patients with premature peripheral arterial occlusive disease

	patients without HHC (162)	patients with HHC[a] (70)	*P*-value
n (%), M/F	90/72(55.6/44.4)	31/39 (44.3/55.7)	0.11
Follow-up (months)	21.8 ± 12.9	24.7 ± 13.0	0.07
Age, y	45.6 ± 6.8	45.8 ± 6.8	0.88
Postmenopausal, *n* (%)	28(17.3)	12(17.1)	0.48
Use of aspirin and/or oral anticoagulants	74(45.7)	26(37.1)	0.25
Cardiovascular history, *n* (%)[b]	33(25.5)	22(31.4)	0.08
Body mass index (kg m^{-2})	24.9 ± 4.2	25.4 ± 4.5	0.51
Current smokers, *n* (%)	125(77.2)	59(84.3)	0.15
Current or past smokers, *n* (%)	145(89.5)	63(90)	0.29
Pack years[c]	27.6 ± 15.1	27.2 ± 14.4	0.79
Hypertension, *n* (%)	40(24.7)	21(30.0)	0.40
Blood pressure (mm Hg)			
Systolic	142.4 ± 19.0	140.6 ± 18.5	0.71
Diastolic	86.3 ± 11.4	85.1 ± 9.0	0.29
Hypercholesterolaemia, *n* (%)	44(27.2)	32(45.7)	0.006
Total cholesterol (mmol L^{-1})	6.1 ± 1.3	6.0 ± 1.1	0.85
LDL cholesterol (mmol L^{-1})[d]	4.2 ± 1.2	4.0 ± 1.0	0.53
HDL cholesterol (mmol L^{-1})	1.1 ± 0.4	1.1 ± 0.3	0.96
Triglyceride (mmol L^{-1})	1.8 ± 1.2	2.1 ± 1.2	0.35
Diabetes mellitus, *n* (%)	14(8.6)	2(2.9)	0.11
Baseline plasma tHcy (μmol L^{-1})			
Fasting	10.3 ± 3.6	17.8 ± 12.7	0.001
Post-methionine	35.4 ± 9.9	74.2 ± 22.6	not tested
Delta[e]	25.9 ± 8.3	56.6 ± 20.8	0.001
After treatment[f]			
Fasting		8.2 ± 2.2	0.001
Post-methionine		36.3 ± 9.4	0.41
Delta		28.5 ± 8.6	0.01
Folate (nmol L^{-1})	9.7 ± 5.6	8.6 ± 4.9	0.14
Vitamin B_6 (nmol L^{-1})	30.8 ± 33.7	27.5 ± 34.6	0.20
Vitamin B_{12} (pmol L^{-1})	258.4 ± 87.2	261.8 ± 93.2	0.94
Creatinine (μmol L^{-1})	84.9 ± 21.5	85.8 ± 21.1	0.73

Data are given as number (*n*) with percentages in parentheses or as mean with SD. HHC, hyperhomocysteinaemia after methionine loading. tHcy, total homocysteine. [a]These patients subsequently received homocysteine-lowering treatment with folic acid and vitamin B_6 (see Methods). [b]Cardiovascular history was defined as a history of angina pectoris, myocardial infarction, transient ischaemic attack and/or stroke. [c]Pack years were calculated by multiplying the number of cigarette packages smoked per day by the number of years the patient smoked. [d]Calculated by Friedewald's formula. [e]Delta indicates the difference between postmethionine and fasting tHcy level. [f]Fasting and postmethionine tHcy levels after treatment were tested against fasting and postmethionine tHcy levels of the patients without HHC.

Table 2 Incidence rates of new cardiovascular events in 232 patients with premature peripheral arterial occlusive disease

	patients without HHC (n = 162)	patients with HHC[a] (n = 70)
Cardiovascular events (n)	0.16 (48)	0.16 (23)[b]
Peripheral arterial occlusive events (n)	0.13 (38)	0.11 (16)
New intermittent claudication (n)	0.014 (4)	0.007 (1)
Progression of peripheral arterial occlusive disease (n)	0.12 (34)[c]	0.10 (15)[c]
Coronary arterial occlusive events (n)	0.024 (7)	0.04 (6)
Angina pectoris or progression of angina pectoris (n)	0.02 (5)[d]	0.03 (5)
Myocardial infarction (n)	0.007 (2)[e]	0.007 (1)
Cerebral arterial occlusive events (n)	0.01 (3)	0.01 (2)
Transient ischaemic attack (n)	0.007 (2)	0.01 (2)
Stroke (n)	0.003 (1)	0
Death[f] (n)	0.007 (2)[g]	0.01 (2)

Incidence rates are expressed as events per person per year. HHC, hyperhomocysteinaemia after methionine loading. n, number of patients. [a]These patients received homocysteine-lowering treatment with folic acid and vitamin B_6 (see Methods). [b]In the hyperhomocysteinaemic group, one patient developed both claudication and angina pectoris at the same time. In Cox-regression analyses this was considered as one cardiovascular event. [c]Of the patients without HHC, 22 had new symptoms after initially successful treatment; 7 had a decrease of walking distance; 4 developed critical ischaemia; and 1 had arterial thrombosis. Amongst the patients with HHC, these numbers were 7, 2, 5, and 1 (see Methods for definitions of these categories). [d]2 patients had progressive angina.
[e]one patient died < 1 month after a myocardial infarction. [f]Three additional patients died; two hyperhomocysteinaemic patients shortly but > 1 month after a new cardiovascular event and one of carcinoma[g].

cular event (Table 2). The crude incidence rate for any cardiovascular event was 0.16 (CI, 0.12–0.20) per person per year in the entire group, 0.16 (CI, 0.12–0.21) per person per year in those with postmethionine normohomocysteinaemia and 0.16 (CI, 0.09–0.22) per person per year in those with postmethionine HHC. The crude RR for the occurrence of cardiovascular events for the hyper- compared to the normohomocysteinaemic patients was 0.96 (CI, 0.59–1.55). The incidence rates of peripheral, coronary or cerebral arterial events did not differ between the groups. Figure 2 shows Kaplan–Meier curves in the normo- and hyperhomocysteinaemic patients groups separately.

Table 3 gives the results of univariate and multivariate Cox-regression analyses. All other risk factors tested (see Statistical analysis) had a P-value >0.2 when considered univariately (data not shown). In multivariate analysis in the entire group of patients, fasting tHcy and triglyceride levels showed significant associations with the incidence of new cardiovascular events. After exclusion of the 35 patients with fasting HHC, the RR for fasting tHcy levels was 1.12 (CI, 0.98–1.27; P = 0.09). In the patients with *normal* postmethionine tHcy levels, multivariate analyses showed significant associations between the incidence of new cardiovascular events and triglyceride levels and both fasting and postmethionine tHcy levels. After exclusion of the 11 patients with normal postmethionine but elevated fasting tHcy levels, RRs for fasting and postmethionine tHcy levels were, respectively, 1.04

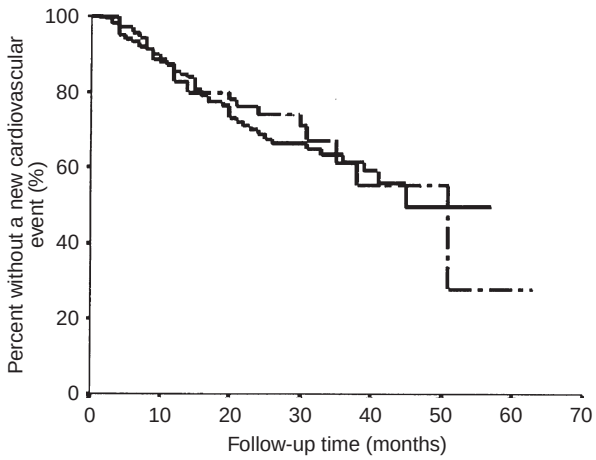

Fig. 2 Kaplan–Meier curves of the occurrence of new cardiovascular events in patients with premature peripheral arterial occlusive disease with normohomocysteinaemia (solid line) and hyperhomocysteinaemia (dashed line) after methionine loading.

Table 3 Cox-regression analysis: risk factors for new cardiovascular events in patients with premature peripheral arterial occlusive disease[a]

	All patients (n = 232)		Patients without HHC (n = 162)		Patients with HHC[b] (n = 70)	
	Relative risk	P-value	Relative risk	P-value	Relative risk	P-value
			Univariate analyses			
Male sex	2.05 (1.25–3.34)	0.004	1.95 (1.06–3.58)	0.03	2.17 (0.93–5.03)	0.07
Cardiovascular history (yes/no)[c]	1.66 (0.98–2.80)	0.06	–	–	2.32 (0.94–5.72)	0.07
Body mass index (per 1 kg m^{-2})	–	–	0.94 (0.86–1.03)	0.19	–	–
Pack years (per 1)[d]	1.01 (0.996–1.03)	0.15	–	–	1.02 (0.99–1.05)	0.19
Diastolic blood pressure (per 1 mmHg)	0.98 (0.95–1.01)	0.17	0.97 (0.94–1.01)	0.11	–	–
Hypercholesterolaemia (yes/no)	–	–	1.86 (1.02–3.40)	0.04	–	–
Total cholesterol (per 1 mmol L^{-1})	1.18 (0.95–1.48)	0.14	–	–	1.47 (0.83–2.60)	0.19
HDL cholesterol (per 1 mmol L^{-1})	0.51(0.21–1.26)	0.15	0.46 (0.17–1.26)	0.13	–	–
Triglyceride (per 1 mmol L^{-1})	1.30 (1.04–1.64)	0.02	1.48 (1.15–1.90)	0.002	–	–
Fasting HHC (yes/no)	–	–	3.20 (1.24–8.23)	0.02	–	–
tHcy levels (per 1 μmol L^{-1})[e]						
Fasting	1.13 (1.06–1.21)	0.0003	1.16 (1.08–1.26)	0.0002	–	–
Post-methionine	1.02 (0.998–1.05)	0.08	1.04 (1.01–1.07)	0.02	–	–
Delta	–	–	1.02 (0.99–1.06)	0.17	–	–
Vitamin B_6 (per 1 nmol L^{-1})	0.98 (0.96–0.99)	0.01	0.98 (0.96–1.002)	0.08	0.97 (0.93–1.004)	0.08
			Multivariate analyses[f]			
Triglyceride (per 1 mmol L^{-1})	1.58 (1.13–2.20)	0.002	1.50 (1.07–2.11)	0.02	–	–
tHcy levels (per 1 μmol L^{-1})[e]						
Fasting	1.20 (1.09–1.34)	0.0004	1.17 (1.05–1.30)	0.006	–	–
Post-methionine	–	–	1.06 (1.01–1.12)	0.02	–	–

HHC, hyperhomocysteinaemia after methionine loading. tHcy, total homocysteine levels. Relative risks (95% confidence intervals) are shown. For the univariate analyses, all RRs with $P < 0.2$ are shown. For the multivariate analyses, all RRs with $P < 0.05$ are shown. [a]Follow-up was (median and range) 20 (1–63) months. [b]These patients received homocysteine-lowering treatment with folic acid and vitamin B_6 (see Methods). [c]Cardiovascular history was defined as a history of angina pectoris, myocardial infarction, transient ischaemic attack and/or stroke. [d]Pack years were calculated by multiplying the number of cigarette packages smoked per day by the number of years the patient smoked. [e]Plasma tHcy levels of the hyperhomocysteinaemic patients are those after treatment (see Methods). [f]Adjusted for all variables with univariate $P < 0.2$ as shown in the upper part of the table.

(CI, 0.88–1.24; P = 0.61) and 1.05 (CI, 0.98–1.15; P = 0.16). When both fasting and postmethionine tHcy were included in the original model, only the association with fasting tHcy remained significant (RR, 1.17; CI, 1.05–1.31). If we then forced postmethionine tHcy into the model, the RR was 1.02 (CI, 0.95–1.08; P = 0.63) for postmethionine and 1.14 (CI, 0.98–1.33; P = 0.08) for fasting tHcy. In the patients with *postmethionine* HHC, there were no significant associations between new cardiovascular events and baseline variables or with post-treatment fasting (adjusted RR, 1.01; CI, 0.76–1.33; P = 0.94) or postmethionine tHcy levels (adjusted RR, 1.01; CI, 0.95–1.07; P = 0.73).

The adjusted RR for the occurrence of cardiovascular events for the patients with HHC, compared to those with normohomocysteinaemia after methionine loading, was 0.76 (CI, 0.33–1.74).

During the follow-up period, newly diagnosed hypertension and hypercholesterolaemia occurred in, respectively, 2.4 and 2.4% of the hyperhomocysteinaemic patients compared to 5.1 and 6.3% (respectively) of the normohomocysteinaemic patients. In addition, 11.9% of the hyperhomocysteinaemic patients stopped smoking and 4.8% started smoking. In the normohomocysteinaemic patients, 21% stopped smoking and 1.3% started smoking. These changes and the changes in treatment of patients with hypercholesterolaemia and/or hypertension did not differ significantly between the groups. Multivitamin use at baseline or during follow-up was infrequent (2 patients).

Discussion

Our study is the first to prospectively investigate the

effects of homocysteine-lowering treatment with vitamin B_6 (250 mg) plus folic acid (5 mg) on the natural course of atherothrombotic disease in patients with premature peripheral arterial occlusive disease and postmethionine HHC. Our data show that, during an average follow-up of 2 years, the cardiovascular prognosis of patients with post-methionine HHC treated with vitamin B_6 and folic acid was similar to or, in the adjusted analysis, slightly better than that of (untreated) patients with normal postmethionine tHcy levels.

Our study population consisted of consecutive patients with premature peripheral arterial occlusive disease referred to a clinical vascular surgery service. In agreement with previous experience, we found a high prevalence of postmethionine and fasting HHC, as well as other cardiovascular risk factors [1, 6–9, 21, 22]. In the whole group, the crude incidence rate for any cardiovascular event after a mean follow-up period of about 2 years was 0.16 per person per year. About 75% of these events involved progression of peripheral arterial occlusive disease; the remaining 25% consisted of coronary and cerebral artery disease (Table 2). Three studies in elderly patients (mean age 61–67 years versus 46 in the present study) found rates of coronary and cerebral artery disease that were similar to ours, and rates of progression of peripheral artery disease that varied from being much lower than in the present study [16, 23] to being comparable [17]. Taken together, these data suggest that our study population is representative of patients with premature peripheral arterial occlusive disease.

Only two prospective studies have investigated clinical outcomes of *untreated* hyperhomocysteinaemic vascular patients. These studies showed that hyperhomocysteinaemic patients, compared to those with normohomocysteinaemia, had a two- to four-fold incidence of cardiovascular morbidity and mortality [17, 18]. Our data show that the cardiovascular prognosis of patients with premature peripheral arterial disease who have HHC after methionine loading and are treated with vitamin B_6 and folic acid is similar to, or, if anything, slightly better than, that of comparable patients who are normohomocysteinaemic after methionine loading. We suggest that these findings, taken together, are consistent with a protective effect of treatment with vitamin B_6 and folic acid.

In patients with vascular disease not treated with homocysteine-lowering agents, even slightly elevated tHcy levels have been found to be associated with more severe atherosclerosis [10–12]. In agreement with these studies [10–12], we found that the hyperhomocysteinaemic, when compared to the normohomocysteinaemic, patients had a higher prevalence of coronary and/or cerebral artery disease at baseline (Table 1) and that higher tHcy levels were positively and significantly related to the incidence of new cardiovascular events (Table 3). It is noteworthy that the latter relation was essentially confined to the normohomocysteinaemic patients, because the absence of such a relation amongst the vitamin-treated hyperhomocysteinaemic patients is again consistent with a protective effect of vitamin treatment. In addition, the association of tHcy levels with the incidence of cardiovascular events in the normohomocysteinaemic patients is in line with recent data indicating that such a relationship extends well within the range of homocysteine levels previously considered normal [18]. Finally, our data are consistent with data showing that treatment with vitamin B_6, when accompanied by a decrease in tHcy levels, is associated with a decreased incidence of thromboembolic disease amongst patients with cystathionine-β-synthase deficiency, i.e. with severe HHC [13, 24].

The most important limitation of our study was that the vitamin treatment was nonrandomized and not blinded. Therefore, alternative explanations for our findings must be carefully considered. First, patients with HHC may have had less cardiovascular risk factors at baseline than patients without HHC. This is not supported by the data shown in Table 1, but we cannot exclude that unmeasured other risk factors differed between the groups. Secondly, assessment of new cardiovascular events might have differed between the groups. However, we stress that we used objective criteria, so this appears unlikely. Thirdly, treatment of cardiovascular risk factors such as hypertension and hypercholesterolaemia might have been more intensive in the hyperhomocysteinaemic group. We found no evidence that this was the case. We did not assess whether the patients prescribed vitamin treatment actually took their vitamins. If they did not, however, one would expect an increased incidence of cardiovascular events in the patients with postmethionine HHC, which was not what we found. Finally, and most important, the patients

with postmethionine HHC might have changed their life-style in such a way that it decreased their cardiovascular risk. Although the data on, for example, changes in smoking habits during the follow-up period clearly do not support this notion, we cannot fully exclude it.

It should be emphasized that the present study was performed in patients with premature peripheral arterial occlusive disease, who had a high rate of cardiovascular complications. Therefore, it is not clear whether our results can be generalized to other patient groups. In addition, we cannot exclude that the favourable effects observed in the vitamin-treated group are due to effects of vitamin B_6 and/or folic acid other than homocysteine lowering [25–27]. Our study was too small to address, with sufficient precision, the question whether the fasting or the postmethionine tHcy level is the strongest predictor of an adverse outcome.

In summary, our data support the hypothesis that homocysteine-lowering treatment with vitamin B_6 plus folic acid can reduce the rapid progression of vascular disease in patients with premature peripheral arterial occlusive disease and postmethionine HHC. Randomized controlled trials are necessary to definitively confirm the effects of folic acid plus vitamin B_6 on cardiovascular disease progression in hyperhomocysteinaemic patients with vascular disease and to determine the optimal treatment regimen. Such trials should focus on both fasting and postmethionine tHcy levels [2, 10] and, in view of recent data showing a continuous relation between tHcy levels and risk of vascular disease [2, 18], should probably use definitions of HHC with lower cut-offs, i.e. less conservative than those we used. Until such studies have been performed, we suggest that it is reasonable to consider treatment with folic acid and vitamin B_6 in patients with premature peripheral arterial occlusive disease who also have postmethionine HHC.

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Correspondence: Dr C. D. A. Stehouwer, MD, PhD, Department of Internal Medicine, Academisch Ziekenhuis Vrije Universiteit, De Boelelaan 1117, 1081 HV Amsterdam, The Netherlands (tel.: + 31 20–4440531; fax: + 31 20–4440502; e-mail: cda.stehouwer@azvu nl).